2016 Protocols

16S rRNA Colony Polymerase Chain Reaction

Adapted from: Weisburg et al 1991 (as noted in Babalola et al 2009)

Purpose:

To amplify the 16S rRNA gene of isolated bacterial colonies for sanger sequencing and taxonomic identification.

Required Materials:

- 10x Buffer
- $MgCl_2$
- dNTPS
- Forward and reverse primers
- DMSO (50%)
- Taq polymerase
- ddH_2O

Procedure

1. Pick a single colony from a LB or Cellulose plate and add it to 10 µl of ddH2O water. Repeat for multiple samples
2. Heat samples at 95°C for 10 min, centrifuge samples for 2-3 min. The supernatant is the template for the PCR reaction.
3. Create PCR reactions for each sample according to the table below.

Reagents	Volume/tube (µl)	Conc
10X Buffer	3	10
MgCl2 (mM)	2.4	50
dNTPs (mM)	0.75	10
primer (F) (uM)	0.6	10
primer (R) (uM)	0.6	10
DMSO (50%)	0	50
Taq (U/ml)	0.18	5000
Template	2	
DI H20	20.47	----
Total Volume	30	

4. Place samples in a thermocycler and create a program with the following parameters:
    a. Initial: 94°C for 2 min
    b. Denature: 94°C for 1 min
    c. Anneal: 58°C for 30 sec for 35 cycles
    d. Extensions: 72°C for 1 min
    e. Final Extension: 72°C for 10 min